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Research Note

Detection of *Campylobacter* from Poultry Carcass Skin Samples at Slaughter in Southern Italy

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ABSTRACT

Campylobacter is a major foodborne pathogen responsible for acute gastroenteritis characterized by diarrhea that is sometimes bloody, fever, cramps, and vomiting. *Campylobacter* species are carried in the intestinal tracts of mammals and birds, and sources of human infection include raw milk, contaminated water, direct contact with pets, and foods, particularly poultry. *Campylobacter jejuni* and *C. coli* are the species that account for the majority of human infections. The aim of this work was to determine the prevalence of *Campylobacter* in 190 poultry carcasses sampled at slaughter and to use a multiplex PCR assay to determine if the isolates were *C. jejuni* or *C. coli*. *C. coli* was not isolated, while *C. jejuni* was recovered from 52 (37.1%) of 140 carcasses for which pools of four sampling sites (neck, cloaca, breast, and back) were examined. In the remaining 50 carcasses, the four sites were analyzed separately, and *C. jejuni* was recovered from the samples in the following order: neck ($n = 20$), cloaca ($n = 16$), breast ($n = 14$), and back ($n = 11$). The results are in agreement with those of other studies, which showed that *C. jejuni* is more commonly associated with poultry than is *C. coli*. Control strategies for *Campylobacter* should include interventions to eliminate *C. jejuni* in poultry at various stages of production and processing, including at slaughter.

Thermophilic *Campylobacter* species are important causes of foodborne illnesses, and current epidemiological evidence suggests that *Campylobacter jejuni* and *C. coli* are the thermophilic species most frequently associated with human gastroenteritis *(4, 6, 19)*. The principal foods associated with outbreaks of human campylobacteriosis are poultry and poultry products *(9, 18)*. However, *Campylobacter* spp. have been isolated from many different food types, including raw and improperly pasteurized milk, meat, and produce *(11)*. The ability of the pathogen to survive in water systems used in animal husbandry and in processing facilities can result in infection in animals and cross-contamination of carcasses *(9, 23)*.

There are few data from Member States of the European Union on changes in the prevalence of *Campylobacter* in broiler flocks over the years. It is well known, however, that the bacteria can adhere to skin folds and particularly to follicles during slaughter *(3, 5)*. European Community recommendations published in December 2003 *(7)* established that each Member State had to develop monitoring systems to obtain more epidemiological information on the distribution of the pathogen and the role played by foods of animal origin in human illness. The "Report of Task Force on Zoonoses Data Collection on Proposed Technical Specifications for a Coordinated Monitoring Programme for *Salmonella* and *Campylobacter spp.* in Broiler Meat in the EU" focuses on the importance of having an understanding of the presence of *Campylobacter* in broiler meat in the European Union *(8)*. According to the report, fresh broiler meat and meat preparations should be targeted at two different stages in the food chain, i.e., at the slaughter house and at retail.

Differentiation of *C. jejuni* and *C. coli* may be problematic and is often based on hippurate hydrolysis, as *C. jejuni* hydrolyzes hippurate while *C. coli* does not. This test is not always reliable given that previous studies have identified *C. jejuni* isolates that are hippurate negative *(16, 21, 24)*. The use of molecular methods, including PCR, may overcome some of the problems encountered in the identification of *Campylobacter* species and increase our understanding of *Campylobacter* epidemiology *(6)*. The present study was undertaken to gain a better understanding of the prevalence of *Campylobacter* in poultry slaughtered in Southern Italy and to identify the carcass site from which *Campylobacter* is isolated more frequently. In addition, a multiplex PCR assay was used to determine if *Campylobacter* strains isolated from poultry carcasses were *C. jejuni* or *C. coli*.

MATERIALS AND METHODS

Sample collection and isolation of *Campylobacter*. During the period from May 2004 to October 2006, a total of 190 broiler carcasses originating from five farms in the Campania region of

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TABLE 1. *PCR primers targeting* Campylobacter *genus and* C. jejuni *and* C. coli *species*

Species targeted	Product size (bp)	Primer name	Sequence (5′–3′)	Reference
C. coli/C. jejuni	400	cadF2B	TTGAAGGTAATTTAGATATG	*12*
		cadR1B	CTAATACCTAAAGTTGAAAC	
C. coli	894	COL1	ATGAAAAAATATTTAGTTTTTGCA	*10*
		COL2	ATTTTATTATTTGTAGCAGCG	
C. jejuni	159	C-1	CAAATAAAGTTAGAGGTAGAATGT	*26*
		C-4	GGATAAGCACTAGCTAGCTGAT	

Southern Italy and slaughtered in the same European Community–recognized slaughter house were investigated. On each of 19 different sampling days, 10 broiler carcasses were analyzed. Sections of skin (2.5 by 2.0 cm, 2.5 g) were taken, after carcass washing and before cooling, with a sterile blade from the neck, cloaca, breast, and back of each carcass randomly sampled at the beginning, at midday, and at the end of the slaughtering day. The four skin samples (10 g total) from each of 140 broiler carcasses were pooled for the analyses, and those from the remaining 50 carcasses were analyzed separately to investigate the prevalence of the bacteria in the different sites.

All samples were immediately transferred to the laboratory in an insulated box and examined on the same day of sampling. The skin was taken aseptically, minced with sterile scissors, and transferred to sterile bags (BagFilter P, 190 by 300 mm; Interscience, Saint Nom La Bretèche, France) containing blood-free enrichment medium (Oxoid, Milan, Italy) at a ratio of 1 to 10. After incubation at 42°C for 24 h under aerobic conditions *(22),* a 10-μl loopful of the culture was streaked onto the surface of modified charcoal cefoperazone deoxycholate agar (Oxoid) and incubated for 48 h at 42°C under microaerobic conditions by using the CampyGen System (Oxoid). Presumptive *Campylobacter* colonies were counted, restreaked onto blood agar plates (Oxoid), and incubated at 37°C for 24 h. Gram and motility tests and catalase and oxidase reactions were used for preliminary speciation. Further confirmation was achieved using the API Campy (bioMérieux, Rome, Italy).

Multiplex PCR analysis. Reference strains of *C. jejuni* CUUG18265 and *C. coli* CUUG11283 were obtained from the Istituto Superiore di Sanità in Rome, Italy. For PCR analysis, *Campylobacter* reference and isolated strains were cultivated on Mueller-Hinton plates (Difco, Becton Dickinson, Milan, Italy) at 42°C for 24 h under microaerobic conditions. Colonies from each plate were suspended in 1 ml of sterile distilled water and pelleted at 16,000 × *g* for 10 min. To extract the DNA, the pellets were resuspended in 200 μl of PrepMan reagent (Applied Biosystems, Monza, Italy), heated at 100°C for 10 min, and then cooled on ice for 2 min. Samples were subsequently centrifuged at 16,000 × *g* for 5 min, and 100 μl of the supernatant, containing the extracted DNA, was removed for PCR analysis. Extracted DNAs from reference strains of *C. jejuni* and *C. coli* were used as positive controls for each assay.

Amplification reactions were performed in total volumes of 50 μl in a Robocycler 96 (Stratagene, La Jolla, CA) containing 5 ng of purified template DNA, 1× PCR Buffer Plus (Invitrogen, Milan, Italy), 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 μM each of the four deoxynucleoside triphosphates, 1.2 U of *Taq* polymerase (Invitrogen), and 0.4 μM each of the primers (Table 1). Primers cadF2B and cadR1B amplify a 400-bp fragment of the *cadF* gene encoding a *C. jejuni* and *C. coli* membrane protein that promotes binding to intestinal epithelial cells *(12).* COL1 and COL2 primers were used to identify *C. coli.* They yield a 894-bp fragment of the *ceuE* gene *(10)* that encodes a 34.5- to 36.2-kDa lipoprotein component of a binding-protein-dependent transport system for the siderophore enterochelin. To identify *C. jejuni,* a fragment of genomic DNA of 159 bp was amplified using C1 and C4 primers *(26).* Samples were subjected to a denaturation step at 94°C for 4 min, followed by 30 amplification cycles, each consisting of 1 min at 94°C, 1 min at 52°C, and 1 min at 72°C, with a final primer extension step of 72°C for 5 min. PCR products were subjected to electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and photographed.

Statistical analyses. The percentages of positive samples from each of the four sampling sites were compared with each other and to that of the pooled samples by using a chi-square test computed using the PROC FREQ procedure of the SAS software package.

RESULTS AND DISCUSSION

Amplification of DNA from reference strains yielded two bands of approximately 400 and 159 bp for *C. jejuni* and two bands of 400 and 894 bp for *C. coli.* Multiplex PCR applied to reference strains enabled the identification of the genus *Campylobacter* and the differentiation of the two species, *C. jejuni* and *C. coli,* as demonstrated by the presence of different-sized product bands (Fig. 1). Differentiation between these two closely related species is important particularly for epidemiological purposes and for treatment of illness *(10, 13).*

All of the strains isolated were *C. jejuni* (Fig. 2); *C. coli* was not isolated. These results are in line with those reported from a national level study by Soncini et al. *(22),* who examined carcass rinses at slaughter, but not in line with results obtained by others, who isolated *C. coli* from 19.3 to 47.5% of chicken samples *(14, 17).* For example, Manfreda et al. *(14)* also examined carcass rinses and identified 47.5% of the isolates as *C. coli.* Parisi et al. *(17)* tested cloacal swabs from broilers and found that of 140 samples, 64 (45.7%) contained *C. jejuni* and 28 (25.7%) contained *C. coli.* Prencipe et al. *(20)* tested raw chicken meat homogenates and found *C. jejuni* in 81.9% of the samples and *C. coli* in 32.5%, and more than one species was isolated from 23.1% of the samples. Testing for thermophilic *Campylobacter* spp. in humans, chickens, and crows, Mdegela et al. *(15)* found that in 341 samples, 91.2% of *Campylobacter* strains from chickens (cloacal swabs) were *C. jejuni* and 8.8% were *C. coli.* Likewise, testing cecal contents of crows showed that 93.8% of 16 isolates were *C. jejuni* and 6.2% were *C. coli*; 96.6% of 59 *Campylobacter* strains isolated from patients with gastrointestinal illness were *C. jejuni.*

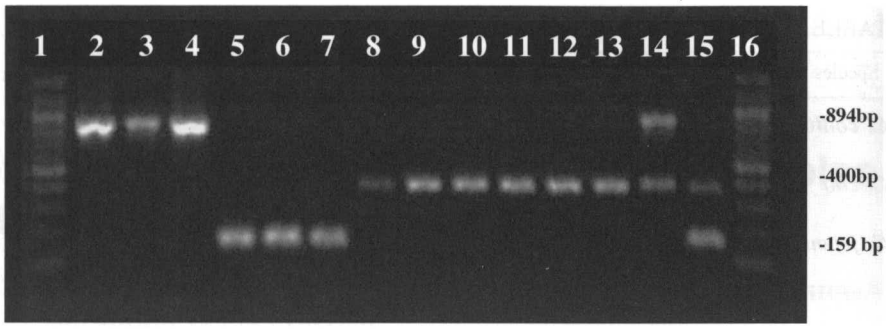

FIGURE 1. *Reference strains tested by singleplex and multiplex PCR assays. Lanes 1 and 16, 100-bp ladder (Invitrogen); lanes 2 to 4,* C. coli *(primers COL1-COL2, 849 bp); lanes 5 to 7,* C. jejuni *(primers C1-C4, 159 bp); lanes 8 to 10,* C. coli *(primers CADF-CADR, 400 bp); lanes 11 to 13,* C. jejuni *(primers CADF-CADR, 400 bp); lane 14,* C. coli *(primers COL1-COL2/CADF-CADR/C1-C4); lane 15,* C. jejuni *(primers COL1-COL2/CADF-CADR/C1-C4).*

In the present study, *C. jejuni* was recovered from pools of four sampling sites in 52 (37.1%) of the 140 examined carcasses (Table 2). In the additional 50 carcasses for which the four skin samples were examined separately, *C. jejuni* was isolated from 20 (40.0%) of the neck, 16 (32.0%) of the cloaca, 14 (28.0%) of the breast, and 11 (22.0%) of the back skin samples. Pairwise comparisons of the percentage of positive results from each of the skin samples were made to determine if differences showed evidence of being significant. The only comparison that came close ($P = 0.52$) was the comparison of the neck (40% positive) and the back skin (22% positive) samples. Furthermore, there were no statistically significant differences between the percentage of positive pooled samples (37.1%) and each of the single skin type samples, except that the percentage of back skin samples (22%) almost showed evidence of significance at P values of 0.5 ($P = 0.051$). Overall, *C. jejuni* was recovered from eight poultry carcasses (16%) from all of the four sampling sites, five carcasses (10%) from the neck and cloaca, four carcasses (8%) from the neck and breast, and two carcasses (4%) from the neck and back. One poultry carcass (2%) was positive for neck, cloaca, and breast, another for cloaca and breast, and one for cloaca and back (Table 2). This is the first study to analyze skin samples for *Campylobacter* from four different areas of chicken carcasses and to compare the results with those of pooled samples. Furthermore, there are very few reports in the literature on the prevalence of *Campylobacter* species from chicken carcasses from slaughter houses in Southern Italy.

According to the Report of the Task Force on Zoonoses Data Collection *(8),* it is important to obtain data both at the slaughter house, to collect information on the contamination level of *Campylobacter* during broiler meat production, and at retail, to know the exposure of consumers to *Campylobacter* via broiler meat. Results obtained at the slaughter house confirm poultry carcasses as a source of *C. jejuni,* underscoring the need to adopt good manufacturing practices to reduce the prevalence of the microorganism, particularly on some sites of the carcass, such as neck and cloaca, where isolation occurred more frequently.

The data on *Campylobacter* prevalence and distribution may be useful at the national and community levels to improve control measures. Microbiological criteria for foodstuffs have been recently reviewed in the European Union; however, Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs *(2)* includes criteria for *Salmonella* in broiler meat but not for *Campylobacter.* The Regulation, however, is open to ongoing review, and microbiological criteria for broiler meat may be considered in the future, when more data will be available, to enable the best characterization of health risk for consumers. In addition, control measures against *Campylobacter* may be considered at different stages of production. Programs for the control of *Salmonella* in flocks of breeding hens and broilers are progressively being implemented according to Regulation (EC) No. 2160/2003 *(1)* and are expected to have an impact on *Salmonella* contamination levels in broiler meat.

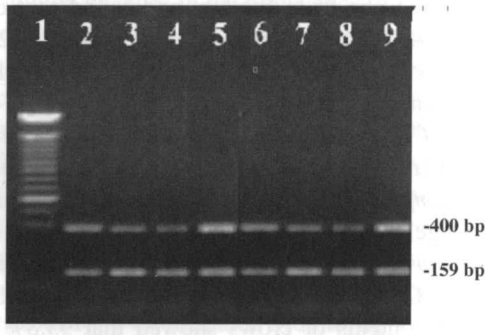

FIGURE 2. *Multiplex PCR assay results for* C. jejuni *poultry isolates. Lane 1, 100-bp ladder (Invitrogen); lanes 2 to 8,* C. jejuni *poultry isolates; lane 9,* C. jejuni *reference strain.*

TABLE 2. *Populations of* C. jejuni *isolated from different poultry carcass sampling sites*

Sample site(s)	No. of carcasses examined	No. (%) positive for *C. jejuni*
Pool of skin[a]	140	52 (37.1)
Skin of neck	50	20 (40.0)
Skin of cloaca	50	16 (32.0)
Skin of breast	50	14 (28.0)
Skin of back	50	11 (22.0)
Skin of neck, cloaca, breast, and back[b]	50	8 (16.0)
Skin of neck and cloaca[b]	50	5 (10.0)
Skin of neck and breast[b]	50	4 (8.0)
Skin of neck and back[b]	50	2 (4.0)
Skin of neck, cloaca, and breast[b]	50	1 (2.0)
Skin of cloaca and breast[b]	50	1 (2.0)
Skin of cloaca and back[b]	50	1 (2.0)

[a] Each pool consisted of four skin samples taken from one carcass.
[b] Data for sample site combinations.

In summary, the current study showed that *C. jejuni* is the *Campylobacter* species found in poultry from the area of Italy examined, and the multiplex real-time PCR assay can be used to distinguish *C. jejuni* from *C. coli*. Furthermore, the neck and cloaca of chickens may be more appropriate sites for sampling of poultry for *Campylobacter*; however, additional studies to confirm these findings are warranted. Furthermore, good manufacturing practices should be designed to ensure a reduction in the prevalence of *C. jejuni* in poultry.

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